

# Your complete source for Western blotting

Load protein samples and MW marker positive controls onto the PAGE gel of choice. Separate proteins through electrophoresis

Transfer proteins from the PAGE gel and immobilize them on the membrane of choice

Stain the membrane to confirm that protein bands have transferred; then destain

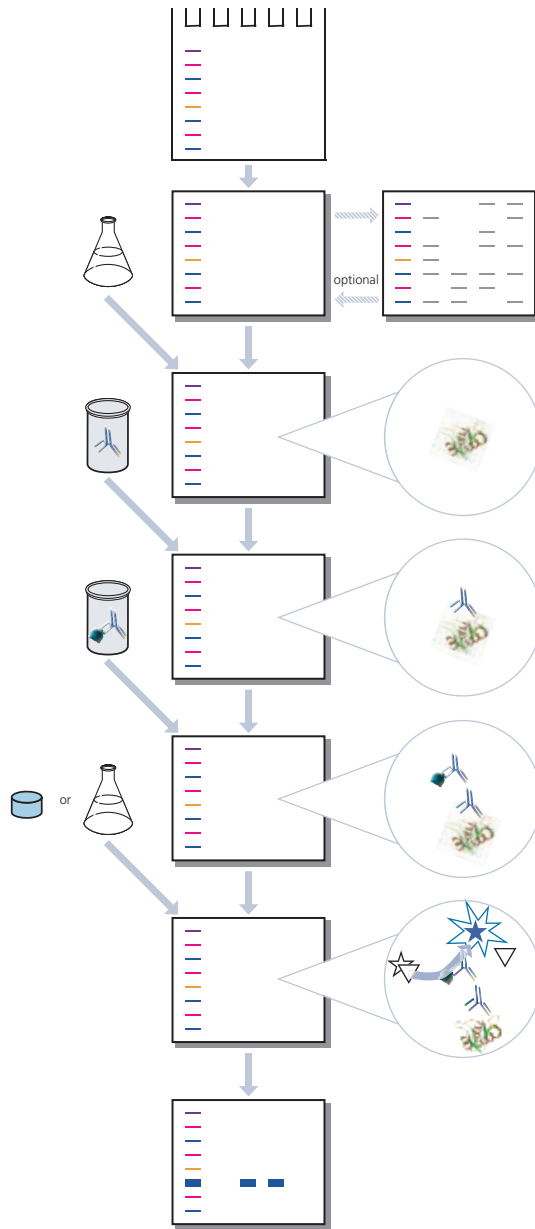
Apply blocking buffer to prevent nonspecific binding

Apply primary antibody specific to the protein of interest

Apply secondary antibody enzyme conjugate specific to the primary antibody

Apply enzymatic substrate to be converted by the conjugate

Observe protein bands of interest



See Chemichrome Western Control and other MW Markers on Pages 10-12

See Transfer Buffers on Pages 13-14

See Membranes and Blotting Paper on Page 17

See Membrane Stains on Page 13

See Western Blocker, other Blocking Buffers and Blocking Reagents on Page 14

See Primary Antibodies in the Sigma General Catalog, the Sigma Life Science Catalog or our web page: [sigma-aldrich.com/antibody](http://sigma-aldrich.com/antibody)

See Antibody-Enzyme Conjugates on Page 7

See CPS, TMB, BCIP/NBT and other substrates (liquid and tablets) on Pages 5-6

See ProteoQwest™ Chemiluminescent Western Blotting Kit on Page 2

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## Western Blotting Kits



### Features & Benefits

- **Includes Chemichrome Western Control**  
Confirms transfer efficiency and acts as positive control for your blot
- **Blots 12 mini-gel (10 x 10 cm) membranes**
- **Ultra-high sensitivity/low background**  
Higher chemiluminescent performance levels locate low abundance proteins
- **Includes qualified buffers and conjugated antibody**  
Confidence in component compatibility and results

### ProteoQwest™ Chemiluminescent Western Blotting Kit, CPS Substrate

Product Code: PQ 0201  
For Mouse Monoclonal IgG Antibodies

ProteoQwest Chemiluminescent Western Blotting Kit with CPS Substrate is designed for high sensitivity chemiluminescent detection of as little as 0.03 ng of protein. It uses the popular Horseradish Peroxidase (HRP) enzyme, and includes essential reagents and antibodies used in a Western blot specific for mouse monoclonal IgG. The chemiluminescent reaction occurs directly on the protein immobilized membrane. This kit is optimized for use with either film or chemiluminescent imagers. All components of the ProteoQwest Chemiluminescent Western Blotting Kit have been extensively tested and optimized to ensure superb results. This kit is designed for 12 mini-gel (10 x 10 cm) blots. Use 0.125 ml per cm<sup>2</sup> of membrane.

### Kit Components

- 200 µl vial of Chemichrome™ Western Control (C 4236)
- 400 ml bottle of Western Blocker Solution (W 0138)
- 12 packets of pre-blended reagents that reconstitute to 500 ml of Tris buffered saline with 0.05% Tween 20 (TBST), pH8 (T 9447)
- 75 µl vial of rabbit anti-mouse IgG (whole molecule) horseradish peroxidase conjugated antibody (A 5225)
- 80 ml bottle of Chemiluminescent Reaction Buffer (C 9232)
- 40 ml bottle of Chemiluminescent Reagent (C 9107)

Chemiluminescent detection of a FLAG®-tagged protein (BAP control protein) in an *E. coli* lysate using Sigma CPS-1 and the top three competitors' products:



### Helpful Hints:

- Aggregate formation in the HRP can cause speckling; filter the conjugate through a 0.2 µm filter.
- Azide is an inhibitor of HRP; be sure to not use azide as preservative.

## Western Blotting Kits *cont.*

### ProteoQwest™ Colorimetric Western Blotting Kit, TMB Substrate

Product Code: PQ 0101  
For Mouse Monoclonal IgG Antibodies

ProteoQwest Colorimetric Western Blotting Kit, TMB Substrate is designed for high sensitivity colorimetric detection of as little as 0.15 ng. The kit includes all qualified essential reagents and antibodies used in a Western blot specific for mouse monoclonal IgG antibodies. The colorimetric reaction occurs directly on the membrane. No darkroom or film is needed when performing a Western blot with the ProteoQwest Colorimetric Kit. This kit is designed for 25 mini-gel (10 x 10 cm) blots.

### Kit Components

- 200 µl vial of Chemichrome™ Western Control (C 4236)
- 2 x 400 ml bottles of Western Blocker Solution (W 0138)
- 25 packets of pre-blended reagents that reconstituted to 500 ml of Tris buffered saline with 0.05% Tween 20 (TBST), pH 8.0 (T 9447)
- One 75 µl vial of rabbit anti-mouse IgG (whole molecule) horseradish peroxidase conjugated antibody (A 5225)
- 100 ml bottle of TMB Substrate for peroxidase detection on membranes [3,3',5,5'-Tetramethylbenzidine] (TMB) Liquid Substrate System for membranes (T 0565)



### Features & Benefits

- **Includes Chemichrome Western Control**  
Confirms transfer efficiency and acts as a positive control for your blot
- **Blots 25 mini-gel (10 x 10 cm) membranes**
- **High-sensitivity**  
Detect at chemiluminescent levels with lower cost
- **Includes qualified buffers and conjugated antibody**  
Confidence in component compatibility and results



**Sigma PVDF membrane (0.15 ng sensitivity)**  
The FLAG® BAP control was used to show how a typical Western blot would be performed with a control. 20 to 0.15 ng of FLAG-BAP was detected on PVDF. No other bands besides FLAG-BAP and Chemichrome IgG (heavy chain) band were detected.

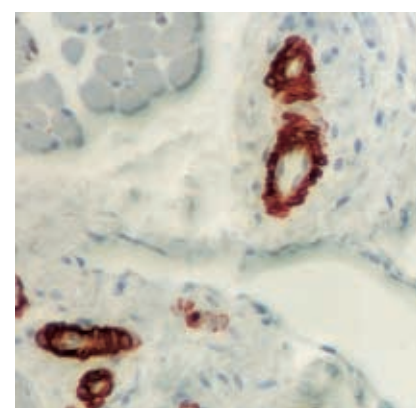


**Sigma nitrocellulose membrane (0.3 ng sensitivity)**  
The FLAG-BAP control was used to show how a typical Western blot would be performed with a control. 20 to 0.3 ng of FLAG-BAP was detected on nitrocellulose. No other bands besides FLAG-BAP and Chemichrome IgG (heavy chain) band were detected.

## Western Blotting Kits *cont.*



The FLAG-BAP control was spiked into an *E. coli* extract and then separated on a 4-20% SDS-PAGE gel along with the Chemichrome western control. The gel was transferred onto a PVDF membrane. The membrane was developed with the anti-FLAG M2 antibody and the reagents for the ProteoQuest Colorimetric Western Blotting Kit with the BCIP/NBT substrate. The FLAG-BAP protein was detected to 0.5 ng with no detection of any *E. coli* proteins.



### ProteoQwest™ Colorimetric Western Blotting Kit, BCIP/NBT Substrate

Product Code: PQ0111

For Mouse Monoclonal IgG Antibodies

ProteoQwest Colorimetric Western Blotting Kit, BCIP/NBT Substrate is designed for high sensitivity colorimetric detection for as little as 0.25 ng. This kit includes all qualified essential reagents and antibodies used in a Western blot specific for mouse monoclonal IgG antibodies. This kit is designed for 25 mini-gel (10 x 10 cm) blots.

#### Kit Components

- 200 µl vial of Chemichrome™ Western Contol (C 4236)
- 25 packets of pre-blended reagents that reconstitute to Tris buffered saline with 3% milk blocking solution (T 8793)
- 25 packets of pre-blended reagents that reconstitute to 500 ml of Tris buffered saline with 0.005% TWEEN 20 (TBST), pH 8.0 (T 9447)
- One vial of anti-mouse IgG alkaline phosphatase conjugated antibody (A 5225)
- 100 ml of BCIP/NBT premixed liquid substrate (B 6404)

### ExtrAvidin® Kits

ExtrAvidin, a unique form of avidin available only through Sigma, facilitates high specificity and low background detection at physiological pH. Comprised of universal reagents for use with primary antibodies, ExtrAvidin staining kits conveniently improve protein detection in a variety of applications, including Western blotting, immunohistology and ELISA. Each kit contains 3 ml of ExtrAvidin-alkaline phosphatase or ExtrAvidin-peroxidase and 3 ml of biotinylated secondary antibodies, specific to primary antibodies of choice, as well as an optimized protocol and complete instructions. The biotinylated antibodies contain a spacer, which improves accessibility for the ExtrAvidin conjugates. Affinity-isolated antibodies in the featured kits have been adsorbed with human IgG and IgM to minimize cross-reactivity.

Product Code	Description	Available Sizes
EXTRA-2	Mouse ExtrAvidin Peroxidase Staining Kit	1 kit
EXTRA-2A	Mouse ExtrAvidin Alkaline Phosphatase Staining Kit	1 kit
EXTRA-3	Rabbit ExtrAvidin Peroxidase Staining Kit	1 kit
EXTRA-3A	Rabbit ExtrAvidin Alkaline Phosphatase Staining Kit	1 kit

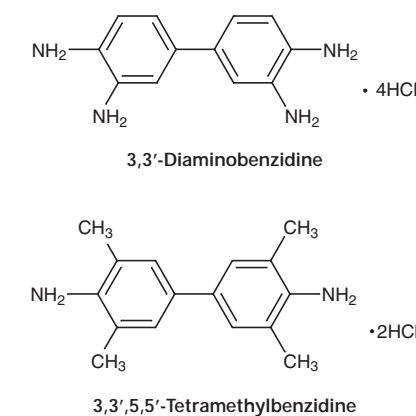
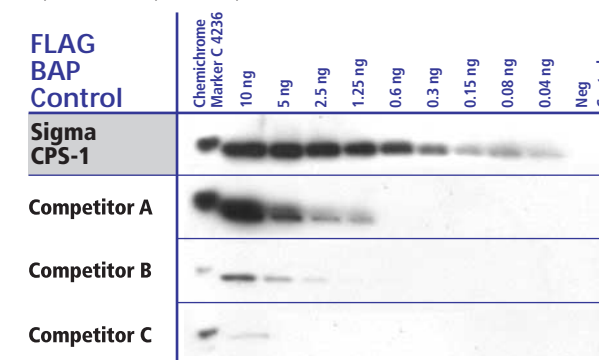
## Peroxidase Substrates

### Chemiluminescent Peroxidase Substrate

Product Code: CPS-1

This two-component liquid peroxidase substrate is included with the ProteoQwest kit (PQ0201), but is also available individually in pack sizes of 60 ml, 120 ml and 300 ml. Use 0.125 ml per cm<sup>2</sup> of membrane.

Chemiluminescent detection of a FLAG®-tagged protein (BAP control protein) in an *E. coli* lysate using Sigma CPS-1 and the top three competitors' products:



#### Helpful Hints:

- Aggregate formation in the HRP can cause speckling; filter the conjugate through a 0.2 µm filter.
- Azide is an inhibitor of HRP; be sure not to use azide as preservative.

### Peroxidase Substrates for Western Blotting

Substrate	Product	Product Code	Color	Comments
TBM	TMB Liquid Substrate for membranes	T 0565	Deep Blue	TMB (3,3',5,5'-Tetramethylbenzidine) represents a safer, more stable alternative to DAB. TMB develops a permanent, insoluble, dark blue reaction product. Product T 0565 is recommended for membrane applications, but not ELISA (microwell) procedures. This product is supplied ready-to-use.
DAB	Sigma FAST™ DAB Tablets	D 4418	Brown	DAB (3,3'-Diaminobenzidine) is more sensitive than AEC. Sigma FAST DAB tablets have been developed for use in immunohistology as well as Western blotting and require no additional buffers or steps to prepare an active substrate solution. Each tablet set dissolved in 15 ml of deionized water yields a ready-to-use buffered solution containing DAB and urea hydrogen peroxide.
	DAB Liquid Substrate	D 7304	Brown	DAB (3,3'-Diaminobenzidine) is more sensitive than AEC. The DAB Liquid Substrate System is recommended for immunohistology as well as Western blotting applications, but not for ELISA (microwell) procedures. DAB Liquid Substrate System includes 25 ml of 10x DAB Liquid Chromagen and 250 ml of ready-to-use buffer/peroxide solution.
	Sigma FAST DAB with Metal Enhancer Tablets	D 0426	Brown	DAB (3,3'-Diaminobenzidine) with Metal Enhancer is more sensitive than both AEC and DAB without metal enhancer. Sigma FAST DAB with Metal Enhancer Tablets require no additional buffers or steps to prepare an active substrate. The DAB reaction is enhanced by the addition of CoCl <sub>2</sub> , and produces a more intense stain which is resistant to alcohol. Each tablet set dissolved in 5 ml of deionized water yields a ready-to-use buffered solution.
CPS	Chemiluminescent Peroxidase Substrate	CPS-1	Chemiluminescent	Chemiluminescent Peroxidase Substrate offers picomole detection, up to 100-fold improvement in signal-to-noise ratio over other luminol-peroxidase formulations.
AEC	AEC Staining Kit	AEC-101	Red	The AEC (3-Amino-9-ethylcarbazole) Staining Kit contains universal reagents for staining peroxidase labeled compounds in Western blotting and immunohistochemistry. AEC produces an insoluble end product and is red in color. The AEC kit is not recommended for ELISA (microwell) procedures. Each kit contains 3 ml of concentrated acetate buffer, AEC chromagen and hydrogen peroxide in a dropper bottle for easy dispensing.
4-Chloro-1-naphthol	4-Chloro-1-naphthol Tablets	C 6788	Blue	4-Chloro-1-naphthol is recommended for Western blotting. This substrate produces an end product that is blue in color and can easily be observed visually.
	4-Chloro-1-naphthol Solution	C 8302	Blue	

## Phosphatase Substrates

### Alkaline Phosphatase Substrates for Western Blotting

Substrate	Product	Product Code	Color	Comments
Fast Red	Sigma FAST Fast Red Tablets	F 4523	Red	Sigma FAST Fast Red TR/ Naphthol AS-MX Tablets have been developed for use in immunohistology and Western blotting applications. Sigma FAST Fast Red TR/ Naphthol AS-MX Tablets contains 0.6 mM levamisole to block endogenous alkaline phosphatase activity. Each tablet set dissolved in 10 ml of deionized water yields a ready-to-use buffered solution.
BCIP/NBT	BCIP/ NBT Liquid Substrate Systems	B 1911	Purple	BCIP/ NBT is more sensitive than Fast Red. BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate dipotassium/Nitrotetrazolium blue chloride) produces an insoluble, dark purple end product for immunohistochemistry and Western blotting applications. This product is not recommended for ELISA (microwell) procedures.
	Sigma FAST BCIP/NBT Tablets	B 5665	Purple	BCIP/ NBT is more sensitive than Fast Red. Sigma FAST BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate dipotassium/Nitrotetrazolium blue chloride) Tablets are commonly used for Western blotting and occasionally for immunohistochemistry. Each tablet dissolved in 10 ml of deionized water yields a ready-to-use buffered solution of BCIP/NBT, pH 9.5.
CDP-Star™	CDP-Star Chemiluminescent Substrate Solution	C 0712	Chemiluminescent	CDP-Star is a sensitive, chemiluminescent substrate allows rapid, reproducible detection of alkaline-phosphatase-labeled molecules in Northern, Southern, and Western blotting applications. Detection of alkaline phosphatase labeled molecules with CDP-Star is extremely sensitive as a result of low background luminescence coupled with high intensity and prolonged light output from the enzyme catalysis. Maximum light emission occurs at approximately 60 minutes and continues for up to 24 hours, allowing for multiple film exposures and/or the sensitive detection of targets present in small amounts. For convenience, CDP-Star is supplied as a 0.24 mM, ready-to-use aqueous solution (i.e. no dilution is necessary). CDP-Star functions on both neutral and positively-charged nylon, giving the reagent added application versatility.

CDP-Star is a trademark of Tropix, Inc. Bedford, MA, USA and covered under U.S. Patent 5,326,882 and 4,931,569.

## Antibody Conjugates

Sigma boasts a broad offering of antibody conjugates including peroxidase, alkaline phosphatase, biotin, avidin, ExtrAvidin, and streptavidin conjugates specific to biological sources as well as particular protein tags. Peroxidase-antibody conjugates are popularly employed in Western blotting due to the wide range of available substrates and the intensity of substrate precipitates. Horseradish peroxidase is a 40 kDa glycoprotein,

optimally active at pH 6.0-6.5, though stable from pH 4 to pH 10. It exhibits thermal stability up to 60 °C and is inhibited by azide. Alkaline phosphatase conjugates are often chosen when greater sensitivity is required. Alkaline phosphatase is a 140 kDa homodimer, optimally active at pH 9.5-10.5. To ensure optimal performance, antibodies are tested prior to conjugation for specificity and immunoreactivity by ELISA.

### Peroxidase Conjugates

Product Code	Name	Label	Features	Pkg. Size
A 5420	<b>Anti-Goat IgG (whole molecule)</b> , Source: Rabbit	HRP	Binds all goat Igs.	1 ml
A 9452	<b>Monoclonal Anti-Goat/Sheep IgG</b> , Clone No. GT-34, Source: Mouse IgG1	HRP	Binds goat G1 and IgG2, sheep and bovine IgG.	1 vial
A 9917	<b>Anti-Mouse IgG (Fab specific)</b>			
A 3682	<b>Anti-Mouse IgG (Fab specific)</b> , Source: Goat	HRP	Binds all mouse Igs. Adsorbed to reduce background staining with the bovine, horse and human samples.	1 ml
A 0168	<b>Anti-Mouse IgG (Fc specific)</b> , Source: Goat	HRP	Binds mouse IgG; does not bind other mouse Igs. Adsorbed to reduce background staining with human samples.	1 ml
A 2554	<b>Anti-Mouse IgG (Fc specific)</b> , Source: Goat	HRP	Binds mouse IgG; does not bind other mouse Igs. Adsorbed to reduce background staining with bovine, horse, or human samples.	1 ml
A 9044	<b>Anti-Mouse IgG (whole molecule)</b> , Source: Rabbit	HRP	Binds all mouse Igs.	2 ml
A 1949	<b>Monoclonal Anti-Rabbit IgG (γ-chain specific)</b> , Clone No. RG-96, Source: Mouse IgG1	HRP	Binds rabbit IgG; does not bind other rabbit Igs.	1 vial
A 9169	<b>Anti-Rabbit IgG (whole molecule)</b> , Source: Goat	HRP	Binds all rabbit Igs.	2 ml
A 0545	<b>Anti-Rabbit IgG (whole molecule)</b> , Source: Goat	HRP	Binds all rabbit Igs. Adsorbed to reduce background staining with human samples.	1 ml
A 2074	<b>Monoclonal Anti-Rabbit Immunoglobulins</b> , Clone No. RG-16, Source: Mouse IgG1	HRP	Binds only rabbit Igs. Does not react with reduced rabbit Igs.	1 vial
P 8651	<b>Protein A</b>	HRP	Binds IgG only from most mammals, except rat, goat, sheep.	500 µg 1 mg 2 mg
P 8170	<b>Protein G, Recombinant</b>	HRP	Binds IgG only from most mammals, except cat; binds chicken IgG.	250 µg 1 mg

## Antibody Conjugates *cont.*

### Alkaline Phosphatase Conjugates

Product Code	Name	Label	Features	Pkg. Size
A 4062	<b>Anti-Goat IgG (whole molecule)</b> , Source: Rabbit	AP	Binds all goat Igs. Adsorbed to reduce background staining with human samples.	0.25 ml 0.5 ml 1 ml
A 4187	<b>Anti-Goat IgG (whole molecule)</b> , Source: Rabbit	AP	Binds all goat Igs.	0.25 ml 0.5 ml 1 ml
A 8062	<b>Monoclonal Anti-Goat/Sheep IgG</b> , Clone No. GT-34, Source: Mouse IgG1	AP	Binds goat IgG1 and IgG2, sheep and bovine IgG.	0.2 ml 0.5 ml
A 2179	<b>Anti-Mouse IgG (Fab specific)</b> , Source: Goat	AP	Binds all mouse Igs. Adsorbed to reduce background staining with bovine, horse or human samples.	0.25 ml 0.5 ml 1 ml
A 1682	<b>Anti-Mouse IgG (Fab specific)</b> , Source: Goat	AP	Binds all mouse Igs. Adsorbed to reduce background staining with human or rat samples.	0.25 ml 0.5 ml 1 ml
A 2429	<b>Anti-Mouse IgG (Fc specific)</b> , Source: Goat	AP	Binds mouse IgG; does not bind other mouse Igs. Adsorbed to reduce background staining with bovine, horse or human samples.	0.25 ml 0.5 ml 1 ml
A 3562	<b>Anti-Mouse IgG (whole molecule)</b> , Source: Goat	AP	Binds all mouse Igs.	0.25 ml 0.5 ml 1 ml
A 2556	<b>Monoclonal Anti-Rabbit IgG (γ-chain specific)</b> , Clone No. RG-96, Source: Mouse IgG1	AP	Binds rabbit IgG; does not bind other rabbit Igs.	0.2 ml 0.5 ml
A 2306	<b>Monoclonal Anti-Rabbit Immunoglobulins</b> , Clone No. RG-16, Source: Mouse IgG1	AP	Binds only rabbit Igs. Provides reduced background with human, guinea pig, rat, bovine, turkey, chicken, goat, sheep, horse, dog, pig or cat samples.	0.2 ml 0.5 ml
A 3687	<b>Anti-Rabbit IgG (whole molecule)</b> , Source: Goat	AP	Binds all rabbit Igs.	0.25 ml 0.5 ml 1 ml
A 3812	<b>Anti-Rabbit IgG (whole molecule)</b> , Source: Goat	AP	Binds all rabbit Igs. Adsorbed to reduce background staining with human samples.	0.25 ml 0.5 ml 1 ml

#### Helpful Hint:

- Do not use milk to block membranes when using an avidin-biotin system; milk contains biotin.

## Antibody Conjugates *cont.*

### Other Conjugates

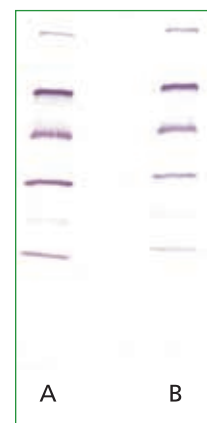
Product Code	Name	Label	Features	Pkg. Size
B 7024	<b>Anti-Goat IgG (whole molecule)</b>	Biotin		
B 7151	<b>Anti-Mouse IgG (Fab specific)</b>	Biotin		
B 0529	<b>Anti-Mouse IgG (Fab specific)</b>	Biotin		
B 7410	<b>Anti-Mouse IgG (Fc specific)</b>	Biotin		
B 9904	<b>Anti-Mouse IgG (Fc specific)</b>	Biotin		
B 8895	<b>Anti-Rabbit IgG (whole molecule)</b>	Biotin		
N 0269	<b>Nickel-Peroxidase Conjugate</b>	HRP	Detects less than 5 ng of polyhistidine tagged fusion protein in microtiter plate based enzyme-linked assays or in Western blot applications.	250 µg
A 5838	<b>Anti-GST-Alkaline Phosphatase Conjugate</b> , Source: Rabbit	AP	IgG fraction of antiserum. By ELISA, the antibody does not recognize GST from rat, rabbit, porcine, or bovine liver or from human placenta.	0.5 ml
A 7340	<b>Anti-GST-Peroxidase Conjugate</b> , Source: Rabbit	HRP	IgG fraction of antiserum. By ELISA, the antibody does not recognize GST from rat, rabbit, porcine, or bovine liver or from human placenta.	0.5 ml
A 8592	<b>Anti-FLAG M2-Peroxidase Conjugate</b>	HRP	Especially useful in detection of FLAG fusion proteins expressed in murine host, where secondary anti-mouse antibodies may cause cross-reactivity.	200 µg 1 mg 5 x 1 mg
A 9469	<b>Anti-FLAG M2-Alkaline Phosphatase Conjugate</b>	AP	Especially useful in detection of FLAG fusion proteins expressed in murine host, where secondary anti-mouse antibodies may cause cross-reactivity.	200 µg 1 mg 5 x 1 mg
A 7058	<b>Monoclonal Anti-polyHistidine-Peroxidase Conjugate</b> , Clone No. HIS-1, Source: Mouse	HRP	Isotype IgG2a. Recognizes synthetic polyHistidine, as well as native or denatured, reduced forms of proteins tagged with 6x Histidines, expressed in selected vectors.	1 vial
A 5588	<b>Monoclonal Anti-polyHistidine-Alkaline Phosphatase Conjugate</b> , Clone No. HIS-1, Source: Mouse	AP	Isotype IgG2a. Recognizes synthetic polyHistidine, as well as native or denatured, reduced forms of proteins tagged with 6x Histidines, expressed in selected vectors.	0.5 ml
P 8651	<b>Protein A - Peroxidase Conjugate</b>	HRP		0.5 ml 1 mg 2 mg
P 8170	<b>Protein G - Alkaline Phosphatase Conjugate</b>	AP		250 µg 1 mg
A 7284	<b>Avidin - Alkaline Phosphatase Conjugate</b>	AP		10 ml 50 ml 500 ml
A 3151	<b>Avidin - Peroxidase Conjugate</b> , Source: Egg Whites	HRP		250 µg 500 µg 1 mg 2 mg 5 mg
A 7419	<b>Avidin - Peroxidase Conjugate</b> , Source: Egg Whites	HRP		2 ml
E 2636	<b>ExtrAvidin - Alkaline Phosphatase Conjugate</b> , Source: Egg Whites	AP		0.2 ml 0.5 ml 5 x 0.5 ml
E 2886	<b>ExtrAvidin - Peroxidase Conjugate</b> , Source: Egg Whites	HRP		0.2 ml 1 ml 5 x 1 ml
S 2890	<b>Streptavidin - Alkaline Phosphatase Conjugate</b> , Source: <i>Streptomyces avidinii</i>	AP		250 µg 1 mg
S 5512	<b>Streptavidin - Peroxidase Conjugate</b> , Source: <i>Streptomyces avidinii</i>	HRP		50 µg 100 µg 250 µg 500 µg 1 mg 2 mg
S 9420	<b>Streptavidin - Peroxidase Polymer Conjugate</b> , Source: <i>Streptomyces avidinii</i>	HRP		250 µg

## Protein Molecular Weight Markers

Diversity, quality, and convenience have earned Sigma's protein molecular weight markers a reputation for excellence. Protein molecular weight markers are used to calculate sample molecular weights, to monitor the progress of an electrophoretic run, or as a positive control for analysis conditions. Sigma offers a

wide selection of markers for numerous protein electrophoresis applications, including silver staining, isoelectric focusing, fluorescent studies, and many more. All have been use-tested to assure outstanding performance and are available in convenient package sizes.

Protein	Subunit MW (kDa)	Biotinylated MW Markers		Fluorescent MW Markers		ColorBurst™	Chemichrome™
		B 2787	SDS-6B	F 3526	F 3401	C 4105	C 4236
Violet protein-dye conjugate	220,000					■	■
Myosin, rabbit muscle	205,000			■			
α <sub>2</sub> -Macroglobulin	180,000	■					
β-Galactosidase, <i>E. coli</i>	116,000	■		■			
Recombinant marker protein or Pink protein-dye conjugate	100,000					■	■
Phosphorylase b, rabbit muscle	97,000	■	■				
Albumin, bovine serum	66,000			■			
Blue protein-dye conjugate	60,000					■	■
IgG marker	59,500						■
Catalase, bovine liver	58,100	■	■				
Ovalbumin, chicken egg or Pink protein-dye conjugate	45,000					■	■
Alcohol dehydrogenase	39,800	■	■	■	■		
Orange protein-dye conjugate	30,000					■	■
Carbonic anhydrase	29,000	■	■	■	■		
Trypsin inhibitor, soybean or Blue protein-dye conjugate	20,100	■	■	■	■	■	■
Lysozyme, egg white	14,300	■	■				
α-Lactalbumin, bovine milk	14,200				■		
Pink protein-dye conjugate	12,000					■	■
Blue protein-dye conjugate	8,000					■	■
Aprotinin, bovine lung	6,500	■			■		



SDS-6B was separated on a 12.5% SDS-PAGE gel and transferred to PVDF (A) and nitrocellulose (B). The proteins were detected using Streptavidin-Peroxidase (S 5512) and 4-chloro-1-naphthol (C 8890).

### Biotinylated Markers

Biotinylated molecular weight markers contain biotin-conjugated proteins which, following electrophoresis and membrane transfer, can be easily detected with a Streptavidin-Peroxidase Conjugate (S 5512) and a color development reagent such as tetramethylbenzidine (TMB, T 0565).

Product Code	Description	MW Range (Da)
B 2787	Biotinylated SDS Molecular Weight Standard Mixture	Wide (6,500-180,000)
SDS-6B	Biotinylated SDS Molecular Weight Standard Mixture	Low (14,300-97,400)

These are both supplied in vials, each sufficient for 2,000 applications (on 10 x 10 cm mini-gels).

## Protein Molecular Weight Markers *cont.*

### Chemichrome™ Western Control

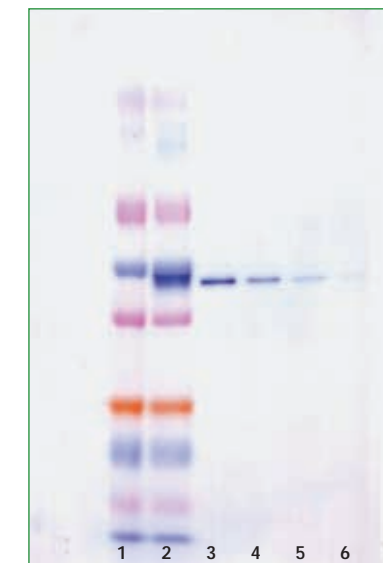
Product Code: C 4236

Chemichrome Western Control is an ideal molecular weight marker and positive control for electrophoresis and subsequent Western blotting. Similar to ColorBurst™, Chemichrome contains an additional band of mouse IgG. During electrophoresis, brightly colored protein bands serve as positive controls for protein migration. During blotting, the same brightly colored bands indicate that the transfer is complete. After incubation

with mouse primary and secondary antibodies, the band of mouse IgG (heavy chain) serves as a positive control using either colorimetric or chemiluminescent substrates. Simple to use and extremely practical, Chemichrome Western Control enables researchers to confirm the success of their analyses every step of the way. Chemichrome is supplied in vials containing 200 µl of ready-to-use solutions.



ColorBurst (lane 1, undetected), Chemichrome (lane 2), and FLAG®-BAP concentrations of 80 ng, 40 ng, 20 ng and 10 ng (lanes 3-6 respectively) were run on a 6-15% Tris-Acetate gel, transferred to PVDF and developed with a chemiluminescent peroxidase substrate (CPS-1) for approximately 5 seconds.



ColorBurst (lane 1), Chemichrome (lane 2), and FLAG®-BAP concentrations of 80 ng, 40 ng, 20 ng and 10 ng (lanes 3-6 respectively) were run on a 6-15% Tris-Acetate gel, transferred to PVDF and developed with a TMB substrate (T 5065).

### Features & Benefits

- **Confirms** that a membrane transfer is complete
- **Confirms** successful Western blotting conditions
- **Compatible** with many peroxidase and phosphatase substrates, including TMB and CPST™

### Fluorescent Markers

Sigma fluorescent molecular weight markers are composed of FITC-conjugated proteins, which are well resolved and easily visualized with ultraviolet light in polyacrylamide gels or membranes.

Product Code	Description	MW Range (Da)
F 3526	Fluorescent Molecular Weight Marker	High (20,100-205,000)
F 3401	Fluorescent Molecular Weight Marker	Low (6,500-39,800)

Sold in vials of powder that, when reconstituted with water, are sufficient for at least 25 applications (for 10 x 10 cm mini-gels).

### Helpful Hint:

- Using Chemichrome or another molecular weight marker and staining after electroblotting allows visual evaluation of the transfer efficiency.

## Protein Molecular Weight Markers *cont.*



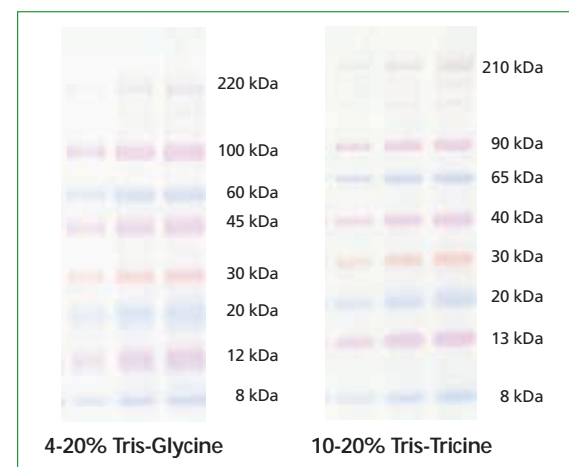
### Features & Benefits

- **Exceptional resolution** allows estimation of sample molecular weights.
- **No freeze/thaw cycle** means decreased degradation and long shelf life.
- **Ready-to-use.** No boiling required before use.
- **No chemical reduction** necessary prior to gel loading.

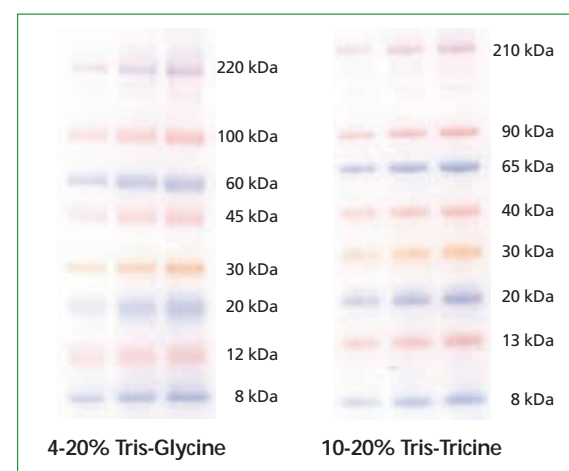
### ColorBurst™ Marker

Product Code: C 4105

Brilliantly colored, exceptionally well resolved, convenient, and stable, ColorBurst protein molecular weight marker performs impressively in a variety of gel compositions and concentrations. ColorBurst is composed of eight proteins ranging from 8 to 220 kDa, which have been chemically reduced, alkylated and conjugated to brilliantly colored dyes. ColorBurst can be used to estimate sample molecular weights, to monitor the progress of an electrophoretic run, or to confirm that an electroblot is complete. Each vial contains 500 ml of marker solution and may be used for up to 100 applications.



Both gels were loaded (left to right) with 3, 5 and 7  $\mu$ l of ColorBurst Marker. The marker was run using standard conditions on 10 x 10 cm, 1 mm thick, 10-well precast gels.



Bands transferred to nitrocellulose membranes from the gels in Figure 1. Transfers were completed in 90 minutes at 70 volts with Towbin's buffer (Tris-Glycine in 20% methanol.) Molecular Masses (kDa) given are apparent values as compared to Sigma's wide range marker set (M 4038). The ColorBurst markers migrate differently with respect to M 4038 in the different gel systems tested.

Product Code	Description	Available Sizes
C 4236	Chemichrome Western Control	1 vial
C 4105	ColorBurst Marker	1 vial

## Buffers, Reagents, & Stains for Western Blotting

### Alcian Blue 8GX

Product Code: A 3157

Alcian Blue 8GX is suitable for detection of glycoproteins on nitrocellulose and in PAGE gels. Alcian Blue 8GX reacts with sulfate and carboxylated functional groups. The interaction of Alcian Blue (cationic) and polyanionic glycoproteins is influenced by pH; both sulfate and carboxylate groups will react at pH 2.5, but sulfate groups alone react at pH 1. Available in 10 g and 25 g sizes.

### Amido Black Staining Solution, 2x concentrate

Product Code: A 8181

Amido Black Staining Solution is designed for rapid staining of protein bands on nitrocellulose membranes. Amido Black Staining Solution facilitates visualization of low concentration proteins with a low background. Proteins can be easily destained with 25% (v/v) isopropanol, and 10% (v/v) acetic acid for further analysis. PAGE gels. Each bottle of 2x concentrate, dilutes to 500 ml with water.

### EZBlue™ Gel Staining Reagent

Product Code: G 1041

Convenient, sensitive, and safe, EZBlue Coomassie Brilliant Blue G-250 colloidal protein stain improves protein electrophoresis results while significantly reducing staining time. Conveniently packaged, EZBlue requires no messy weigh-ups or additions of methanol or acid. As a colloidal stain, it reacts only with proteins, not the gel itself. Background staining is reduced, so protein bands can be visualized almost immediately. No destaining step is required, although a water wash may intensify bands and clarify the background. EZBlue is compatible with both PAGE gels and PVDF membranes. Most impressively, EZBlue is extremely sensitive, detecting as little as 5 ng of protein. Available in 500 ml and 3.8 L sizes.

#### Helpful Hints:

- Sometimes proteins bind poorly to the membrane; add 20% methanol to the transfer buffer to aid binding.
- To improve transfer of high molecular weight proteins efficiency, add SDS to 0.1% to the transfer buffer. Low SDS concentrations in the transfer buffer can improve transfer efficiency but may reduce the membrane's ability to retain some proteins.
- Immerse the filter in glutaraldehyde immediately after transfer. Glutaraldehyde cross-linking of protein improves stability of small acidic proteins for immunoblotting, which could otherwise diffuse out of the membrane. Use 0.2% glutaraldehyde in Tris-buffered saline for 45 minutes.

### Fast Green FCF

Product Code: F 7252

Fast Green FCF is used for protein detection and quantitation in native PAGE, SDS-PAGE, and IEF gels. Following staining, protein concentration can be measured at 625 nm. Fast Green staining is linear over a wider range of detection than Brilliant Blue R. It is especially well suited to IEF because it does not bind to ampholytes. Available in 5 g and 25 g sizes.

### Gold, Colloidal

Product Code: 50755

As a widely used protein and polysaccharide marker for electron microscopy, colloidal gold is a useful general stain for proteins immobilized on nitrocellulose membranes following PAGE. Available in 500 ml size.

### Glycoprotein Detection Kit

Product Code: GLYCO-PRO

The glycoprotein detection kit is designed to selectively stain glycoproteins in polyacrylamide gels and membranes using a modification of the Periodic Acid-Schiff (PAS) methods. Staining of sugar moieties of glycoproteins yields magenta bands with a colorless background. The detection limits using this kit are 25-100 ng of carbohydrate per band. Each kit contains sufficient materials for 10 mini-gels (10 x 10 cm).

### Ponceau S

Product Code: P 3504

Ponceau S (sodium salt) may be used to prepare a stain for rapid reversible detection of protein bands on nitrocellulose or PVDF membranes (Western blotting), as well as on cellulose acetate membranes. Common stain formulations include 0.1% (w/v) Ponceau S in 5% acetic acid or 2% (w/v) Ponceau S in 30% TCA and 30% sulfosalicylic acid. Available in 10 g, 50 g and 100 g sizes.

### Ponceau S Solution

Product Code: P 7170

Ponceau S Solution is a ready-to-use, reversible staining solution designed for rapid (5 minute) staining of protein bands on nitrocellulose or PVDF membranes (Western blots), as well as cellulose acetate membranes. Ponceau S stain is easily reversed with water washes, facilitating subsequent immunological detection. Available in 1 L size.

## Buffers, Reagents, & Stains for Western Blotting *cont.*

### Reversible Protein Detection Kit for Membranes and Polyacrylamide Gels

**Product Code: R-PROB**

The Reversible Protein Detection Kit for Membranes and Polyacrylamide Gels is a unique protein detection product for visualization of proteins on nylon, nitrocellulose, and PVDF membranes, as well as PAGE gels with sensitivity comparable to that of Coomassie stains. Many other stains produce high backgrounds on nylon membranes due to strong charge interactions. Application of activated R-PROB produces lavender-stained protein bands, which are easily destained (reversed) with an EDTA solution. Each kit is sufficient for 200 applications (small blots on 8 x 10 cm PAGE gels).

### SYPRO® Ruby Protein Blot Stain

**Product Code: S 4817**

Fluorescent SYPRO Ruby protein blot stain provides a rapid, simple, and highly sensitive method for detecting proteins on nitrocellulose or polyvinylidene difluoride (PVDF) membranes (blots). The bright orange-red fluorescent stain can be easily visualized using UV illumination or a laser scanner. Available in 200 ml sizes.

*SYPRO is a registered trademark of Molecular Probes.*

### Albumin, Bovine Serum

**Product Code: A 9647**

Available in 10, 50, and 100 g sizes.

### Alkaline Phosphatase Stabilizing Buffer

**Product Code: A 4955**

Specially made for the stable dilution of alkaline phosphatase-antibody conjugates, this solution contains 1% bovine serum albumin and 15 mM sodium azide in Tris-buffered saline, pH 8.0. Available in 100 ml and 1 L sizes.

### CAPS

**Product Code: C 2632**

Used in Western blotting. Available in 25 g, 100 g, 6 x 100 g, and 250 g sizes.

### λ-Carrageenan

**Product Code: C 3889**

This product contains essentially pure lambda carrageenan isolated from two species, *Gigartina aciculaire* and *G. pistillata*. Available in 1, 5, 25, and 100 g sizes.

### Casein Solution, 5%, from Bovine Milk

**Product Code: C 4765**

Blocking solution for Western blotting. Available in a 10 ml size.

### Gelatin from Cold Water Fish

**Product Code: G 7765**

This product contains approximately 45% gelatin in water, as well as 0.15% propyl p-hydroxybenzoate and 0.2% methyl p-hydroxybenzoate as preservatives. Available in a 250 ml and 1 L sizes.

### Gelatin from Porcine Skin

**Product Code: G 8150**

Blocking protein for Western blotting. Available in a 100 g and 500 g sizes.

### Glutaraldehyde Solution, 25% in Water

**Product Code: G 6257**

Available in 10 ml, 10 x 10 ml, 100 ml, and 1 L sizes.

### Glycine

**Product Code: G 8898**

Glycine is a component of Towbin's buffer. Available in 500 g and 1 kg sizes.

### MES hemisodium salt

**Product Code: M 0164**

Contents of each pouch dissolved in 1 liter deionized water will yield 0.1 M sodium MES buffer, pH 6.1 at 25 °C. Available in 10 pack and 5 x 10 pack sizes.

### Methanol

**Product Code: M 1775**

Available in 1 gallon and 4 x 1 gallon sizes.

### Milk, non-fat, dried, bovine

**Product Code: M 7409**

Reconstitution with 100 ml deionized water yields a 3% w/v solution. Available in 1 bottle and 5 bottle sizes.

### MOPS hemisodium salt

**Product Code: M 0289**

Contents of each foil pouch dissolved in 1 liter of deionized water yield 0.1 M sodium MOPS buffer. Available in 10 pack and 5 x 10 pack sizes.

## Buffers, Reagents, & Stains for Western Blotting *cont.*

### Peroxidase Stabilizing Buffer

**Product Code: P 9209**

Specially made for the stable dilution of peroxidase-antibody conjugates, this solution contains 1% bovine serum albumin and an antimicrobial agent in phosphate buffered saline, pH 7.4. Available in 1 L size.

### Phosphate Buffered Saline Tablet, pH 7.4

**Product Code: P 4417**

One tablet dissolved in 200 ml of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4 at 25 °C. Available in 50 tablet and 100 tablet sizes.

### Phosphate Buffered Saline 10x Concentrate

**Product Code: P 7059**

Used in Western blotting. Available in 1 L package size.

### Phosphate Buffered Saline, pH 7.4

**Product Code: P 3813**

Contents of one pouch, when dissolved in one liter of distilled or deionized water, will yield 0.01 M phosphate buffered saline (NaCl 0.138 M; KCl: 0.0027 M); pH 7.4 at 25 °C. Available in 1 pack, 10 pack, and 5 x 10 pack sizes.

### Phosphate Buffered Saline with Bovine Serum Albumin, pH 7.4

**Product Code: P 3688**

Contents of one pouch, when dissolved in one liter of distilled or deionized water, will yield 0.01 M phosphate buffered saline (NaCl: 0.138 M; KCl: 0.0027 M); BSA: 1% w/v, pH 7.4 at 25 °C. Available in 10 pack size.

### Phosphate Buffered Saline with 3% non-fat milk, pH 7.4

**Product Code: P 2194**

Each pouch dissolved in 100 ml deionized water will yield 0.05 M phosphate buffered saline, pH 7.4 at 25 °C, containing 3% w/v non fat milk. Available in 10 pack and 5 x 10 pack sizes.

### Phosphate Buffered Saline with TWEEN 20, pH 7.4

**Product Code: P 3563**

Contents of one pouch, when dissolved in distilled or deionized water, will yield 0.01 M phosphate buffered saline (NaCl: 0.138 M; KCl: 0.0027 M); TWEEN 20: 0.05%, pH 7.4 at 25 °C. Available in 10 pack and 5 x 10 pack sizes.

### Phosphate Citrate Buffer Tablet

**Product Code: P 4809**

One tablet dissolved in 100 ml deionized water yields a 0.05 M phosphate citrate buffer, pH 5.0 at 25 °C. Available in 50 and 100 tablet sizes.

### Phosphate Citrate Buffer with Sodium Perborate Capsule

**Product Code: P 4922**

The contents of one capsule dissolved in 100 ml of deionized water yields 0.05 M phosphate citrate buffer, containing 0.03% sodium perborate as a substitute for H<sub>2</sub>O<sub>2</sub>, pH 5.0 at 25 °C. Available in 50 and 100 capsules sizes.

### Phosphate Citrate Buffer with Urea Hydrogen Peroxide Tablet

**Product Code: P 9305**

Phosphate Citrate buffer tablets with Urea Hydrogen Peroxide may be used as a peroxide substrate. Available in 50 tablet and 100 tablet sizes.

### Sodium Dodecyl Sulfate (SDS)

**Product Code: L 3771**

Available in 25 g, 100 g, 500 g, and 1 kg sizes.

### Tris-Glycine Buffer 10x Concentrate

**Product Code: T 4904**

Tris-glycine buffer is used to make a Tris-glycine-methanol transfer buffer. The 10x Tris-glycine buffer is diluted to 1x with methanol and water to make a solution containing 25 mM Tris, 192 mM glycine, and 20% methanol. Available in 1 L size.

### Tris Buffered Saline Tablets

**Product Code: T 5030**

One tablet dissolved in 15 ml of deionized water yields 0.05 M Tris and 0.15 M sodium chloride, pH 7.6 at 25 °C. Available in 50 and 100 tablet sizes.

### Tris Buffered Saline, pH 8.0

**Product Code: T 6664**

The contents of one pouch, when dissolved in one liter of distilled or deionized water, will yield 0.05 M Tris buffered saline (NaCl: 0.138 M; KCl: 0.0027 M); pH 8.0 at 25 °C. Available in 10 pack size.

## Buffers, Reagents, & Stains for Western Blotting *cont.*

### Tris Buffered Saline with Bovine Serum Albumin, pH 8.0

Product Code: T 6789

Contents of one pouch, when dissolved in one liter of distilled or deionized water, will yield 0.05 M Tris buffered saline (NaCl: 0.138 M; KCl: 0.0027 M); bovine serum albumin: 1% w/v, pH 8.0 at 25 °C. Available in 10 pack size.

### Tris Buffered Saline with 3% non-fat Milk, pH 8.0

Product Code: T 8793

Contents of one pouch, when dissolved in 100 ml of distilled or deionized water, will yield 0.05 M Tris buffered saline, pH 8.0 at 25 °C, containing 3% w/v non-fat milk. Available in 10 pack and 5 x 10 pack sizes.

### Trizma Base

Product Code: T 1503

Available in 15 g, 100 g, and 250 g sizes.

### Urea, 8M

Product Code: U 6504

Available in 500 g and 1 kg package sizes.

### Western Blocker Solution

Product Code: W 0138

Western Blocker solution has been optimized for detection systems using horseradish peroxidase (HRP) conjugates; it is also compatible with alkaline phosphatase based systems. Western Blocker Solution is compatible with both PVDF and nitrocellulose membranes. Available in 400 ml size.

## Membranes & Blotting Paper

### QuickDraw™ Blotting Paper

QuickDraw blotting paper is used for Northern, Southern, and Western blots. Extra-thick paper is ideal for Western blotting to sandwich the gel and the membrane. The thick paper may

be used as a filter support for dot blots. Medium thickness paper is suitable for use as a wick during transfers. QuickDraw paper is available in a variety of sizes to meet your needs.

Product Code	Description
P 6664	Blotting Paper, Medium-Thick, 20 x 20 cm
P 6914	Blotting Paper, Medium-Thick, 30 x 30 cm
P 9039	Blotting Paper, Medium-Thick, 33 x 56 cm
P 9164	Blotting Paper, Thick, 33 x 56 cm
P 6928	Blotting Paper, Extra-Thick, 7 x 8 cm
P 7796	Blotting Paper, Extra-Thick, 7 x 10 cm

Product Code	Description
P 8046	Blotting Paper, Extra-Thick, 10 x 15 cm
P 6803	Blotting Paper, Extra-Thick, 11 x 14 cm
P 8549	Blotting Paper, Extra-Thick, 14 x 20 cm
P 7921	Blotting Paper, Extra-Thick, 15 x 15 cm
P 8171	Blotting Paper, Extra-Thick, 20 x 20 cm
P 7176	Blotting Paper, Extra-Thick, 20 x 25 cm

### Immobilon™-P PVDF Membranes

Immobilon-P PVDF (Polyvinylidene Difluoride) membranes have been optimized for Western blotting and offer better

handling and staining than nitrocellulose. All Immobilon-P PVDF membranes have a pore size of 0.45 mm.

Product Code	Description
P 2438	Immobilon-P PVDF Membrane 9 x 12 cm
P 4188	Immobilon-P PVDF Membrane 10 x 10 cm
P 2563	Immobilon-P PVDF Membrane 15 x 15 cm

Product Code	Description
P 2813	Immobilon-P PVDF Membrane 26 x 26 cm
P 2938	Immobilon-P PVDF Membrane 26.5 cm x 3.75 m

### Nitrocellulose Membranes

Nitrocellulose membranes are the most popular matrix used in protein blotting. All of the following are suitable for Western blotting. Most proteins can be successfully blotted

using a 0.45 µm pore size membrane. For proteins of low molecular weight or peptides, a 0.2 µm pore size membrane is recommended.

Product Code	Description
N 7892	Nitrocellulose Membrane 0.2 µm pore size, 15 x 15 cm
N 8017	Nitrocellulose Membrane 0.2 µm pore size, 20 x 20 cm
N 8142	Nitrocellulose Membrane 0.45 µm pore size, 7 x 10 cm

Product Code	Description
N 8267	Nitrocellulose Membrane 0.45 µm pore size, 15 x 15 cm
N 8392	Nitrocellulose Membrane 0.45 µm pore size, 20 x 20 cm